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Afterpotentials in Dronefly Retinula Cells

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Summary. The wavelength dependence of the afterpotentials following a bright illumination was studied in single photoreceptor cells of the dronefly *Eristalis*. Cells with only a spectral sensitivity peak in the blue were selected. As previously demonstrated, these cells contain a rhodopsin absorbing maximally at about 450–460 nm, which upon photoconversion transforms into a metarhodopsin absorbing maximally at about 550 nm (Tsukahara and Horridge, 1977).

With the visual pigment initially all in the rhodopsin form, a high rate of visual pigment conversion results in an afterhyperpolarization (AHP) when the fraction of metarhodopsin remains negligible after illumination as occurs at longer wavelengths if the intensity is high. Intensive illumination at short wavelengths is followed by a prolonged depolarizing afterpotential (PDA). The magnitude of the PDA peaks at low intensities at about 450–460 nm, corresponding to the peak of the cell's spectral sensitivity (i.e. the rhodopsin peak). With increasing intensity of illumination, however, the peak shifts progressively towards 430 nm, which corresponds to the photoequilibrium with maximum metarhodopsin that can be established by monochromatic light. From this result, it is inferred that the PDA is related to the induced fall in the rhodopsin fraction. The PDA can be abolished, or knocked down, by a long-wavelength flash which reconverts remaining metarhodopsin into rhodopsin. Therefore the decline of the PDA is restrained by the existing amount of metarhodopsin. Possible theories of afterpotentials are discussed.

Introduction

Following intense illumination of the visual sense cells of arthropods two types of afterpotentials have been observed. Firstly, the afterhyperpolarization (AHP)

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has been described for *Limulus* lateral eye (Benolken, 1961; Kikuchi et al., 1962), ventral eye (Brown and Lisman, 1972), dragonfly (Naka, 1961), barnacle (Koike et al., 1971), and honeybee drone (Baumann and Hadjilazaro, 1971). The current theory is that afterhyperpolarization results when Na^+-ions, which have flowed into the visual sense cell during the previous depolarization, are actively transported out (Wulff and Mueller, 1975). Secondly, a prolonged depolarizing afterpotential (PDA) has been reported for *Limulus* median eye UV-receptors (Nolte and Brown, 1972; Minke, Hochstein and Hillman, 1973). On the basis of extensive experiments on barnacle photoreceptors, Hochstein et al. (1973) and Minke et al. (1973) have found that a prolonged depolarizing afterpotential (called by them the tail of the response) is intimately connected to the visual pigment processes that are characteristic of invertebrates, namely, that rhodopsin conversion results in a stable metarhodopsin (rev. Goldsmith, 1972; Hamdorf et al., 1973; Hamdorf and Schwemer, 1975). A high rate of rhodopsin conversion results in a PDA, and photoconversion of excess metarhodopsin during the PDA gives rise to a cancelling (a tail-depressing effect) of this afterdepolarization (Hochstein et al., 1973; Brown and Cornwall, 1975a, b; see also Nolte and Brown, 1972). The term we use for the latter is knock-down effect (KDE).

In this paper we investigate the afterpotentials in the peripheral retinula cells of the dronefly *Eristalis tenax*. The characteristics of the visual pigment are known in some detail (Stavenga, 1976; Tsukahara and Horridge, 1977). The rhodopsin absorbs maximally in the blue with a peak near 460 nm and the metarhodopsin absorbs maximally in the yellow at about 550 nm (Fig. 1). A strongly bathochromic shifted metarhodopsin is typical of flies, and therefore our analysis of after potentials will be relevant to other studies on fly photoreceptors. In fact, the large spectral separation between rhodopsin and metarhodopsin turns out to be methodologically advantageous for discriminating different aspects of the afterpotentials and for correlating them with the visual pigment processes. The afterpotentials in fly visual cells have also been apparent for some time (e.g. Washizu, 1964, in the blowfly), and the prolonged depolarization and the knock-down effect on it have been demonstrated in blowfly (Muijser et al., 1975) and fruitfly (Minke et al., 1975a, b).

An unsolved question related to peripheral photoreceptors of flies is the origin of the high UV-peak of the sensitivity spectrum. Although we have recently provided strong evidence against the view that the UV-peak is caused by a separate UV-absorbing visual pigment in the peripheral retinula cells (Tsukahara and Horridge, 1977), in this present study we have selected cells with a low sensitivity in the UV, so that the spectral characteristics of the afterpotentials can be correlated with the properties of the blue-absorbing rhodopsin and the yellow-absorbing metarhodopsin without additional complications.

We have studied four aspects of the potentials recorded from dronefly photoreceptors;

i) the (late) receptor potential (LRP), i.e. the depolarization caused by illumination at all wavelengths;

ii) the prolonged afterdepolarization (PDA), i.e. the long-lasting depolarization following illumination with intense blue light;

iii) the afterhyperpolarization (AHP), i.e. the hyperpolarization following intense yellow or red light;

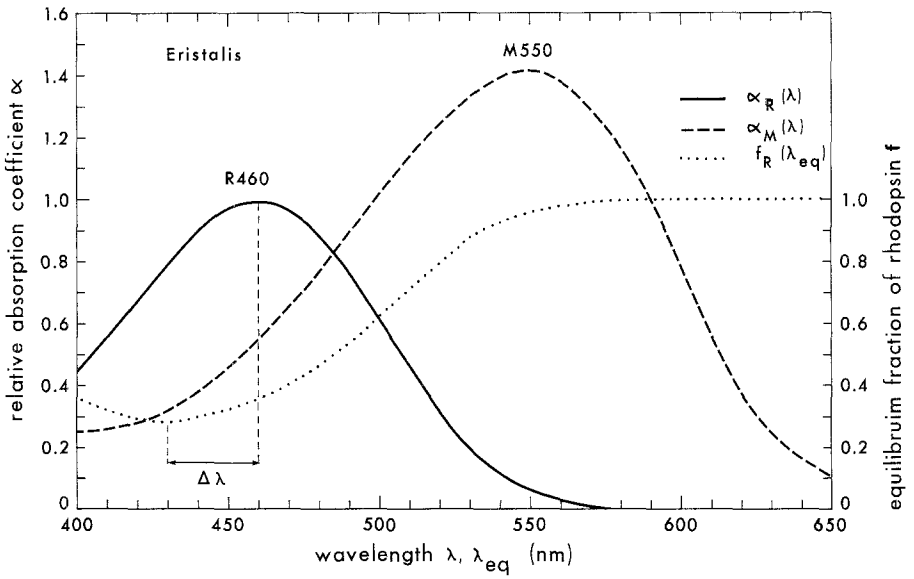

Fig. 1. The relative absorption coefficients of dronefly visual pigments in peripheral retinula cells, from Stavenga (1976). Rhodopsin R 460 is interconvertible with metarhodopsin M 550. Monochromatic light of wavelength λ_{eq} and of sufficiently long duration, establishes a photoequilibrium, where the fraction of molecules in the rhodopsin state is $f_R(\lambda_{eq})$. It is assumed that the quantum efficiency for conversion of rhodopsin into metarhodopsin γ_R is equal to the quantum efficiency of the reverse reaction γ_M, see Equations (1) and (2). The minimum rhodopsin fraction occurs at $\lambda_{eq}=430$ nm, which is $\Delta\lambda=30$ nm less than the wavelength of the rhodopsin peak

iv) the knock-down effect (KDE), i.e. the cancelling of the PDA by yellow or red light;

We shall relate the spectral properties of the potentials to those of the visual pigment (Fig. 1). It has to be understood that blue light effectively converts and removes rhodopsin until a photoequilibrium is established with a low rhodopsin fraction $f_R(\lambda_{eq})$ and a high metarhodopsin fraction $f_M(\lambda_{eq})$. On the other hand, yellow and red light effectively converts metarhodopsin, and shifts the photoequilibrium to a state with a maximum rhodopsin fraction, $f_R(\lambda_{eq})=1$ (see Fig. 1); for details of the dronefly visual pigment see Tsukahara and Horridge (1977).

Methods

Experimental Procedures

Techniques were as described before (Horridge and Mimura, 1975; Horridge et al., 1975). The data reported here are obtained from 3 *Eristalis* retinula cells; each of these was held for several hours during which time a large number of varied measurements could be made. Numerous other cells that could not be held for so long all gave substantially the same results. An essential requirement for maintaining healthy photoreceptor responses proved to be that only a minute hole was made in the eye of the fly and that the animal was allowed to ventilate normally (see Horridge et al., 1975).

The illumination originated in a 900 W Xenon arc which supplied two separately collimated light beams each with sets of neutral density and interference filters. These two beams were brought together

to a single UV-transmitting light guide, 4 mm in diameter, the other end of which was attached to a perimeter arm with a radial distance of 10 cm, subtending about 2° 18′ at the eye. The narrow band interference filters (Schott) were compensated by metallized quartz neutral density filters to pass constant numbers of quanta at 16 wavelengths. Intensity $\log I = 0.0$ corresponds to 4.45×10^{14} quanta $cm^{-2} s^{-1}$ at all wavelengths.

Before each set of measurements it was checked that the stimulus was situated exactly on the optical axis of the cell. A conditioning flash of 5 s and 9×10^{14} quanta $cm^{-2} s^{-1}$ at 574 nm was always used between experimental tests to ensure that approximately all visual pigment molecules were in the rhodopsin state before each stimulus (see Tsukahara and Horridge, 1977). Each test light was given 2 min after the conditioning flash.

Results

The Receptor Potential (LRP)

The response of a photoreceptor cell to a long-lasting light stimulus is an initial phasic peak which falls to a plateau (Fig. 2). The maximum value of the plateau was measured directly after the peak (indicated with *a* in Fig. 3, inset) as a reference for quantifying the afterdepolarization (see below). The spectral sensitivity of a photoreceptor cell, determined from the plateau value (Fig. 4), is in accordance with the general view that the receptor potential is directly related to the photoconversion of rhodopsin.

The Prolonged Afterdepolarization (PDA)

Following the "off" at a high intensity stimulus with light of short wavelength there is a tail on the receptor potential, and with increasing intensity of blue light (451 nm, duration 1 s), the height and the duration of the tail increases (Fig. 2).

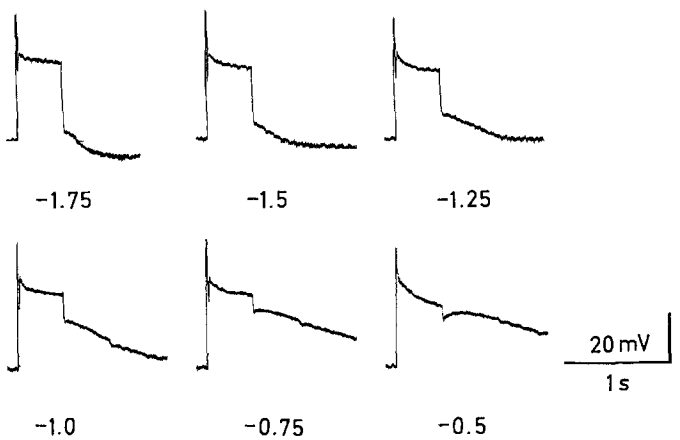

Fig. 2. The development of the PDA as an effect of intensity above that required to saturate the receptor potential. The stimulating flash (wavelength 451 nm, duration 1 s) causes a depolarizing receptor potential. With increasing intensities a PDA develops. The stimulus intensity is indicated below each trace by the value of $\log I$; $\log I = 0.0$ corresponds to 4.45×10^{14} quanta $cm^{-2} s^{-1}$ at the eye surface

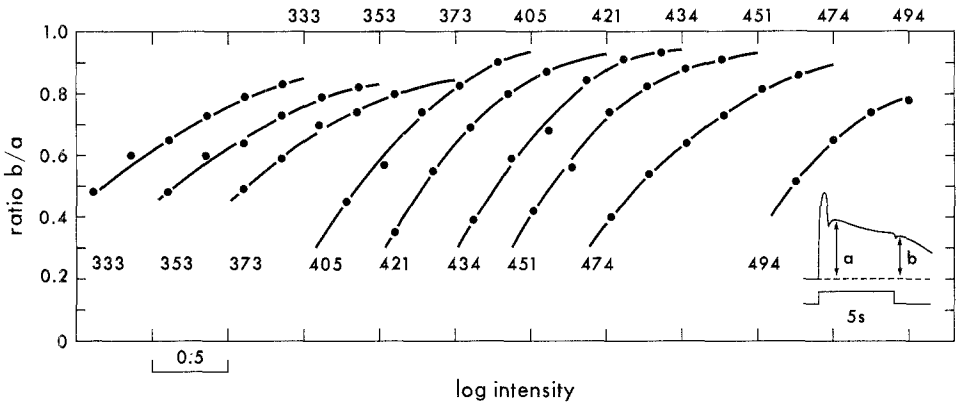

Fig. 3. The ratio of the afterpotential (value *b*, see inset) to the plateau receptor potential (value *a*) as a function of intensity at a variety of wavelengths, all from one cell. For clarity's sake the *b/a*-curves are displaced 0.5 log unit of intensity from each other. The maximum intensity is indicated for each wavelength by the vertical bar below the wavelength value (nm) at the top of the figure. Comparison with Figure 1 shows that the curves are steepest and reach highest at wavelengths where the photoequilibrium rhodopsin fraction is lowest. See further Figure 4

This PDA gradually falls back to the resting potential and must therefore be measured at a defined time after the stimulus. As there is often a small notch in the response at "off", we found the simplest measure of the PDA to be the maximum height after this notch. The height is marked as *b* in Figure 3 inset. As shown in Figure 2 the dynamic range of the PDA is over about 2 log units of intensity above that required to saturate the receptor potential. Because the LRP is saturated, the height *a* of the initial part of the plateau should be constant. We have expressed the height *b* of the PDA as a fraction of the height of the plateau *a* to avoid any possible contaminating effect resulting from minute drift of the resting potential (less than 5 mV/30 min).

To investigate the underlying processes we have analyzed the curves by measuring the value of *b/a* at a number of wavelengths and intensities. Figure 3 shows the ratio *b/a* as a function of intensity from an experimental series similar to that given in Figure 2, for a number of different wavelengths (duration of illumination 5 s). The curves of *b/a* are steepest and reach highest at 434 nm. The same data but with *b/a* plotted as a function of wavelength at each intensity, is presented in Figure 4. At low intensities the magnitude of the PDA peaks at about 450–460 nm, which is near the peak of the spectral sensitivity curve. However, with increasing intensity of illumination, the peaks shift progressively towards 430 nm.

The spectral shift shown in Figure 4 can be interpreted in terms of the photochemistry of the visual pigment. We pose the hypothesis that the prolonged depolarizing afterpotential measured as the *b/a* ratio is determined by the number of converted rhodopsin molecules. Before presenting arguments for this hypothesis we recall that each illumination in the experiments of Figure 2 and 3 has been given with all visual pigment molecules initially in the rhodopsin state. Hence, at low intensity illumination only a small fraction of the molecules is converted.

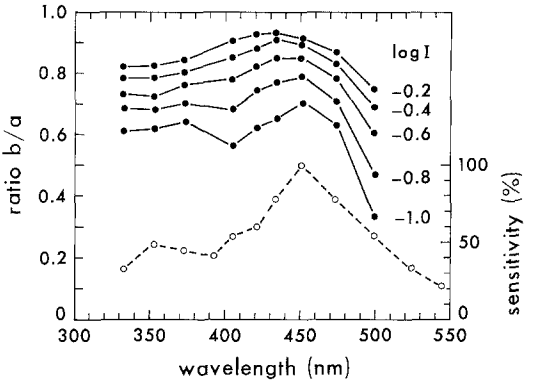

Fig. 4. Ratio of afterpotential (value *b* inset) to receptor potential (value *a*) as a function of wavelength at a variety of intensities (continuous lines). The data are derived from Figure 3. Log $I = 0.0$ corresponds to an intensity of 4.45×10^{14} quanta cm^{-2} s^{-1} (at all wavelengths). The spectral sensitivity of the receptor potential is given by the dashed curve. At the lowest intensities used in the afterpotential experiments (which still saturate the receptor potential), the afterpotential (PDA) and the receptor potential (LRP) peak at similar wavelengths (450–460 nm). At higher intensities the PDA peak shifts towards 430 nm. This shift may be correlated with the increasing amount of metarhodopsin formed (see Fig. 1)

This fraction is the fraction of metarhodopsin molecules formed and is proportional to the absorption coefficient of rhodopsin, or $f_M(\lambda)$ is proportional to $\alpha_R(\lambda)$, following the terminology in Figure 1. So, at low intensities the maximum metarhodopsin fraction will result after illumination at about 460 nm.

On the other hand, at intensities sufficiently high to establish a photoequilibrium within the duration of the illumination, the resulting fraction of metarhodopsin molecules is:

$$f_M(\lambda_{eq}) = 1 \Big/ \left(1 + \frac{\alpha_M(\lambda_{eq})\,\gamma_M}{\alpha_R(\lambda_{eq})\,\gamma_R}\right) = \frac{\alpha_R(\lambda_{eq})\,\gamma_R}{\alpha_R(\lambda_{eq})\,\gamma_R + \alpha_M(\lambda_{eq})\,\gamma_M} \tag{1}$$

where α_R and α_M are the molecular absorption coefficients of rhodopsin and metarhodopsin respectively, and γ_R and γ_M the quantum efficiencies (for further details see Tsukahara and Horridge, 1977). The fraction of metarhodopsin formed at each wavelength can be read from Figure 1. Since:

$$f_M(\lambda_{eq}) = 1 - f_R(\lambda_{eq}) \tag{2}$$

the maximum metarhodopsin fraction results from illumination at about 430 nm at high intensity. At intermediate intensities the maximum metarhodopsin fraction follows illumination at a wavelength between 430 and 460 nm. Therefore, the data of Figure 4 are consistent with the hypothesis that the PDA is correlated with the fraction of metarhodopsin molecules formed.

Knock-down Effect (KDE)

The experimental series of Figure 7 (same cell as Fig. 5) shows the depolarisation created by a 4 s pulse of blue light (434 nm). During the PDA, at 4 s after the first

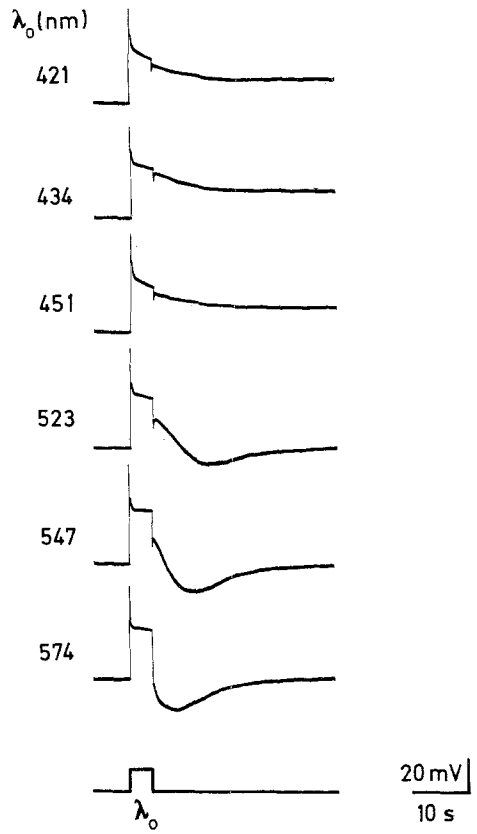

Fig. 5. Afterpotentials in response to flashes of constant duration and equal quantum numbers over a range of wavelengths. The intensity applied ($\log I = -0.15$) saturates the receptor potential at all wavelengths shown. The afterpotential changes gradually from a prolonged depolarizing afterpotential (PDA) into an afterhyperpolarization (AHP)

pulse, another 4 s pulse is given at a selected wavelength. Usually the second pulse induces an additional receptor potential. A PDA remains after a second pulse of short wavelength but after longer wavelengths the PDA is not only knocked down, but sometimes the KDE is noticeable even during the red pulse.

The wavelength-dependence of the knock-down effect of Figure 7 has been plotted in Figure 6. Again the maximal hyperpolarization, but now the one caused by the second pulse, was measured. This is c_{max} and the potential value c was measured on each record for the moment when c_{max} occurred. Values of c/c_{max} were plotted against the wavelength of the second pulse (open circles and solid line in Fig. 6).

The two curves of Figure 6 are similar, although the initial conditions which generate them are quite different. At the onset of the pulses in Figure 5 all visual pigment molecules were in the rhodopsin state ($f_R = 1$, $f_M = 0$), but at the onset of the second pulse in Figure 7 about 30% of the molecules were rhodopsin and 70% metarhodopsin ($f_R = 0.3$; $f_M = 0.7$).

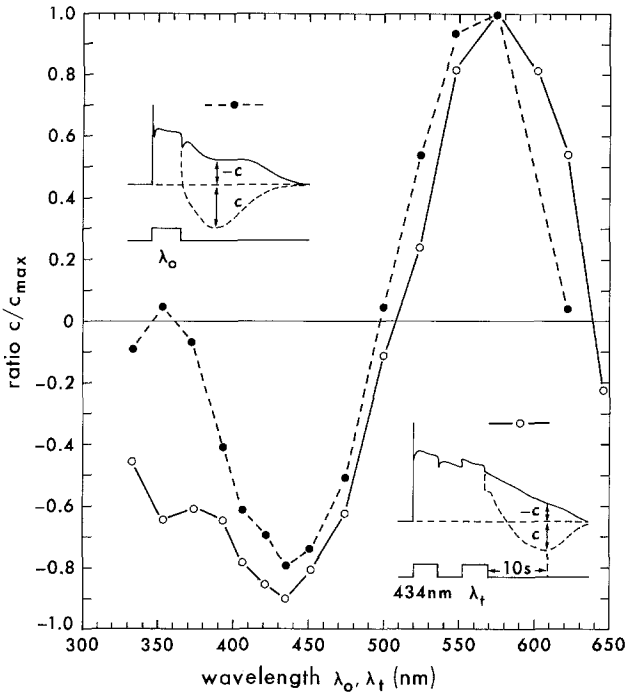

Fig. 6. Dependence of the afterpotential on the wavelength of the single flash (dashed curve) and of the second flash in the double experiment (continuous line). The data are derived from the experimental series shown in Figure 5 and Figure 7, respectively

The Influence of Metarhodopsin

The results of a long series of further experiments similar to those of Figures 5 and 7, and all taken from the same cell, are shown in Figure 8. The recordings were analysed as follows. First, the wavelength dependence of the potential was measured directly after the "off", at b, yielding the curve b/b_{max}, which appears to be essentially similar to the upper curve in Figure 4. Secondly, the time necessary for 30% decrease of the PDA was calculated as shown in the inset, and plotted as τ/τ_{max} in Figure 8. The resulting curve also peaks at about 430 nm. The initial height of the afterpotential and the 30% decay time thus appear to be related.

The effect of the second pulse on the PDA of the same cell is also shown in Figure 8. The potential measured directly after the second pulse is taken as the fraction of the value which the PDA would have had if the second pulse had not been applied. A second pulse at about 430 nm elevates the depolarization still further, and one at about 600 nm maximally suppresses the PDA. All of the curves in Figure 8 favour the view that a high metarhodopsin concentration in some way is correlated with a slow decay of the afterdepolarization. During the PDA, reducing the metarhodopsin content evidently favours a quick recovery to the resting potential.

During the receptor potential, however, reducing the metarhodopsin content

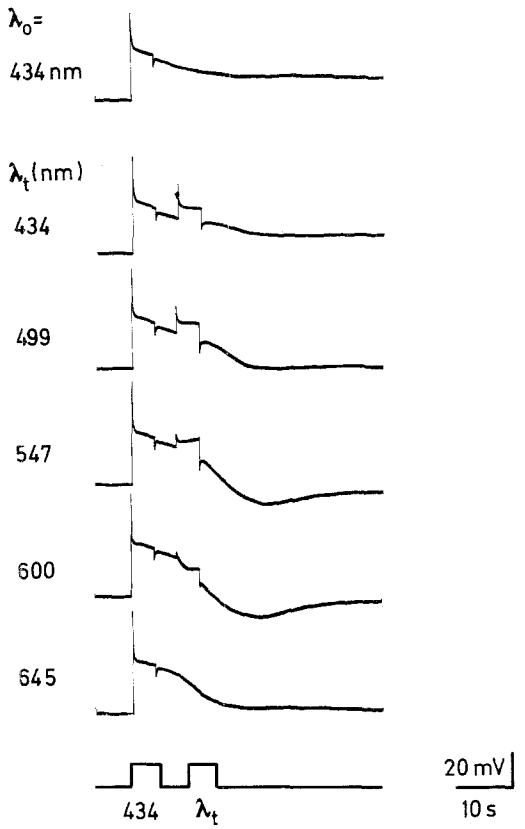

Fig. 7. The effect of a second pulse of selected wavelength λ_t on the PDA created by a 434 nm flash. Duration of flashes and interval is 4 s; intensity of first flash $\log I = -0.15$ and of second flash $\log I = -0.4$. The second flash causes an additional depolarization in all cases except at red wavelengths. The longer wavelengths cancel the created PDA or even give rise to an afterhyperpolarization; same cell as Figure 5. In the case of the single flash experiment the value of the afterpotential c is determined at the point on the record where the maximal hyperpolarization c_{max} occurs. In the case of the double flash experiment the value of c is read at 10 s after the second pulse

does not cause a return to resting potential. The experiment of Figure 9 illustrates this. We adapted with an intense light of 405 nm (4.45×10^{14} quanta $cm^{-2}\ s^{-1}$; duration 30 s) and so established a photoequilibrium with about 35% rhodopsin and 65% metarhodopsin. After 10 min, when the induced PDA had declined and the resting potential was restored, weak flashes (also 405 nm; 4.45×10^{12} quanta $cm^{-2}\ s^{-1}$; 1 s duration) provided a running measure of the sensitivity. Then a long red stimulus was given (600 nm, 4.45×10^{14} quanta $cm^{-2}\ s^{-1}$; 15 s), and we observe that the resulting receptor potential increases during the stimulus. An obvious conclusion is that the red light increases the rhodopsin concentration and thus the sensitivity of the cell. This conclusion is reinforced in that the continued test pulses at 405 nm show an enhanced response. Related observations have been made by Hamdorf et al. (1971, 1973) and by Hamdorf and Rosner (1973) on *Ascalaphus* and the blowfly.

Below we will discuss this conclusion and the previous ones, together with data and theories published in a number of recent reports on invertebrate photoreceptors.

An extension of the same hypothesis is that reconverting the metarhodopsin molecules into the native rhodopsin state will abolish the PDA. Metarhodopsin conversion is effectively executed by yellow-red light and such light indeed knocks

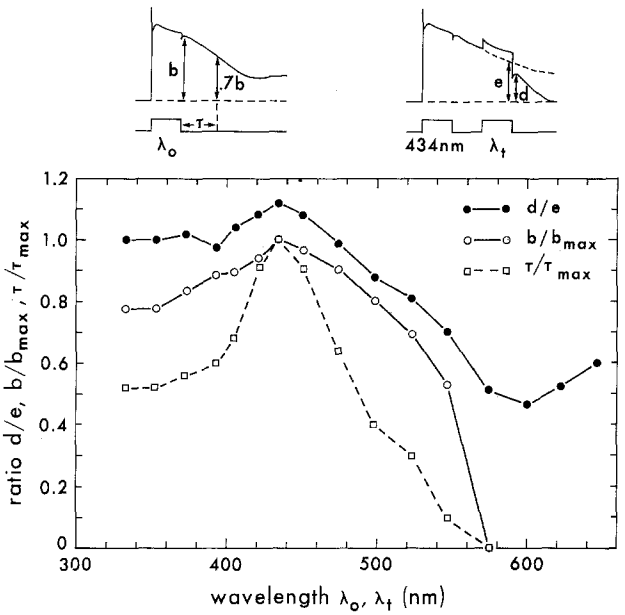

Fig. 8. Afterpotential values at light off and the time for 30% decrease of PDA, all from a single cell. The afterpotential value at light off b is normalized to its maximal value b_{max}, which is attained at $\lambda_0 = 434$ nm. The cell in this single flash experiment was very stable (see Methods). The time τ necessary for the PDA to decay to $0.7b$ is also presented as normalized to the maximal value τ_{max}, which equals 15 s in this particular experiment. The curves shown all peak at 434 nm, not at the peak of spectral sensitivity, supporting the hypothesis that the prolonged depolarizing afterpotential is related to a high metarhodopsin concentration. The afterpotential (value d) at light off in the double flash experiment has been normalized to the extrapolated potential (value e) which the PDA would have taken if the second flash had not been applied

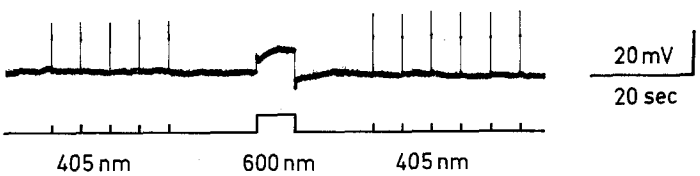

Fig. 9. Recovery of receptor cell sensitivity induced by red light. First the cell was adapted to an intense light (4.45×10^{14} quanta cm^{-2} s^{-1}) of 405 nm for 30 s, giving $f_R(\lambda_{eq}) = 0.35$ (Fig. 1). The sensitivity was then tested with 1 s flashes of wavelength 405 nm (4.45×10^{12} quanta cm^{-2} s^{-1}). The responses to five flashes are shown. Subsequently a red stimulus of 600 nm was given for 15 s (4.45×10^{14} quanta cm^{-2} s^{-1}). This resulted in an increasing depolarization during the stimulus, presumably as a consequence of an increasing rhodopsin concentration. The subsequent response to the 405 nm test flashes reveal a markedly enhanced sensitivity

down the PDA. However, before demonstrating this we first investigate the effect of wavelength on the afterpotential.

Afterhyperpolarization (AHP)

The receptor potential and the afterpotentials resulting from intense illumination at a variety of wavelengths (of equal quantum flux) are shown in Figure 5. At all wavelengths the receptor potential is saturated. The afterpotential after blue light is the prolonged depolarization (PDA), but at longer wavelengths the afterdepolarization progressively inverts into a hyperpolarization.

Measurements from a large series of records similar to Figure 5 have been plotted in Figure 6. The value of the afterpotential c is read off from the recording at the point in time after the stimulus at which the maximum hyperpolarization c_{max} occurs, and the ratio c/c_{max} is given by the dashed line in Figure 6. Clearly, violet-blue wavelengths create a PDA, which is correlated with a high net conversion of rhodopsin into metarhodopsin as discussed above. Green to red wavelengths on the other hand induce an AHP. At those wavelengths only a small or even negligible metarhodopsin fraction results. This can be seen at the right side of Figure 1 where $f_R(\lambda_{eq})$ is large. Even so, an intense yellow illumination does produce a turnover of rhodopsin molecules, which is sufficient to cause a saturated receptor potential. The rate of turnover, however, decreases with further increase in wavelength because the absorption coefficient of rhodopsin falls off rapidly. As is evident from Figures 5 and 6, the afterhyperpolarization diminishes in the red, and hence we may tentatively conclude that an AHP is correlated with the rate of visual pigment turnover. This conclusion is supported by experiments using a variety of intensities at long wavelengths (data not shown).

Discussion

The present study on the afterpotentials in peripheral retinula cells of the dronefly is based on previous microspectrophotometrical (Stavenga, 1976) and electrophysiological investigations (Horridge et al., 1975; Tsukahara and Horridge, 1977). The afterpotentials become conspicuous at illumination intensities which saturate the receptor potential (Fig. 2). Let us first deal with the afterhyperpolarization caused only by long wavelength illumination.

It has been concluded that the AHP observed in drone bee, *Limulus* and barnacle is caused by an electrogenic Na^+ pump (Baumann and Hadjilazaro, 1971; Koike et al., 1971; Brown and Lisman, 1972; see Wulff and Mueller, 1975). A similar conclusion for the drone fly is quite acceptable, since sodium inflow will be substantial at intensities above LRP saturation. Although visual pigment turnover is indeed high, the rhodopsin fraction remains high at long wavelengths owing to the relatively high absorption coefficient of metarhodopsin. Intense illuminations of shorter wavelengths on the other hand result in a substantial reduction in the rhodopsin fraction (Fig. 1) and accordingly the afterhyperpolarization is swamped by the prolonged afterdepolarization (Fig. 5). A similar

transition can be observed when going from lower (but still LRP saturating) intensities to high intensities at a fixed short wavelength (Fig. 2).

The emergence of the PDA is related to a substantial conversion of rhodopsin into metarhodopsin. At low intensities the wavelength dependence of the PDA follows the rhodopsin spectrum (Figs. 1, 4). As concluded by Baumann and Hadjilazaro (1972) in the only previous analysis of insect afterdepolarization, the PDA depends on the same mechanism as the receptor potential. From this, however, we cannot tell whether it is the rate of change in rhodopsin concentration or the rate of conversion of rhodopsin into metarhodopsin which is best correlated with the PDA. To distinguish between these options we tested the effect of intensity. With long or intense stimuli the peak wavelength for generation of the PDA moves to 434 nm. Similarly, Stavenga et al. (1975), in a study of the pupil in blowfly retinula cells, observed that the speed of dark adaptation of the pupil is most delayed by wavelengths in the region where the rhodopsin fraction decrease is extreme. Even so, because the total decrease in rhodopsin during illumination equals the amount of metarhodopsin at light-off, the PDA may well be caused by some process which accumulates during the stimulus.

In the case of *Limulus* UV-receptors, three pieces of evidence favour the decrease in the rhodopsin fraction and not the residual metarhodopsin at "off" as the cause of the PDA (Minke et al., 1973). Firstly, the afterdepolarization decays slowly over 20 min but the metarhodopsin fraction does not change correspondingly. Secondly, conversion of metarhodopsin into rhodopsin has a short-lived inhibitory effect on the PDA of subsequent responses. Thirdly, when the afterdepolarization has decayed spontaneously, applying the same stimulus as before does not produce a new afterdepolarization, apparently because the rhodopsin-metarhodopsin equilibrium at that wavelength has already been attained.

Therefore in *Limulus* the condition required to generate an afterdepolarization is more rhodopsin conversions than metarhodopsin conversions, and evidently the metarhodopsin fraction does not uniquely determine the membrane potential. Whether flies have the same mechanism of origin of the PDA as *Limulus* is not yet investigated. The knock-down effect is at first sight caused by a decrease in metarhodopsin fraction. As discussed above, however, experiments on *Limulus* show that the PDA is not predominantly governed directly by the residual metarhodopsin fraction.

An excitor-inhibitor theory has been proposed on the basis of work on the barnacle eye (Hochstein et al., 1973). On this theory, the PDA is a maintained depolarization caused by an excitor substance which is generated by rhodopsin conversion, and the excitor effect is cancelled by an inhibitor generated by metarhodopsin conversion. This theory has been taken up by others, e.g., Minke et al. (1975) and Stark (1975) to interpret the ERG of mutant *Drosophila*, where receptor potentials presumably make the significant contribution. It is apparent from the present study that the spectral dependence of afterpotentials and visual pigment states do not have coincident peaks, contrary to the assumption made by Stark (1975), but that afterpotentials have a more complex relationship to the visual pigment conversions. Furthermore, the study of Minke et al. (1975) is an analysis of the discrepancy between the ERG and intracellular records from cells 1–6, but omits the effect of the ERG upon the intracellular record, which is strongly

dependent on the position of the reference electrode (Tsukahara, unpublished observations). In the white eyed mutant the ERG may reach 30 mV (Minke et al., 1975) because many retinula cells are illuminated simultaneously. Therefore it is essential to use two electrodes close together to measure the true transmembrane potential changes. Both papers assume that the theory based on afterpotentials in barnacle and *Limulus* applies to receptor potentials in fly and make no use of the ratio of the rhodopsin-metarhodopsin fraction.

That the excitor-inhibitor theory meets serious difficulties is clear from the following experiment. After a maintained strong stimulus at any wavelength a further stimulus of the same wavelength of any intensity excites equal numbers of rhodopsin and metarhodopsin molecules, but the response is a typical receptor potential. Therefore there cannot be equal numbers of two intermediate transmitter substances which cancel each other's effects. This observation is even more relevant when responses to stimuli of long wavelength follow a blue conditioning light as shown in Figure 9. At 600 nm the rhodopsin absorption is less than 1% of the absorption by the metarhodopsin. Before the 600 nm stimulus is applied the rhodopsin fraction had been decreased to about 35% by the 405 nm illumination. Even so, although the rhodopsin absorption of the red light is minimal, and the metarhodopsion absorption is relatively enormous, a clear receptor potential occurs. If an inhibitor influencing the membrane potential exists it would certainly have been activated, since the 600 nm illumination during the PDA effectively induces a knock-down effect (Fig. 7). Therefore, we conclude that there cannot be excitor and inhibitor substances having unequally large and opposite effects on the membrane potential. For closely parallel observations on the blowfly, see Stavenga et al. (1975) and Muijser et al. (1975).

In conclusion, we can state that the afterhyperpolarization of dronefly is similar to that in other arthropods but is not always obvious. The prolonged afterdepolarization is correlated hypothetically with a substance which is accumulated upon rhodopsin conversion, and removal of which is delayed when the visual pigment is in the metarhodopsin state. The knock-down effect would then be a controlled removal of this substance. Comparing our work on the dronefly and other insect photoreceptors with the results published by other investigators, we feel that these conclusions can be upheld, but a more extensive scheme cannot be proposed at present because the observed phenomena differ widely among different species.

References

Baumann, F., Hadjilazaro, B.: Afterpotentials in retinula cells of the drone. Vision Res. **11**, 1198 (1971)

Baumann, F., Hadjilazaro, B.: A depolarizing effect of intense light in the drone visual receptor. Vision Res. **12**, 17–31 (1972)

Benolken, R.M.: Reversal of photoreceptor polarity recorded during the graded receptor potential response to light in the eye of *Limulus*. Biophys. J. **1**, 551–564 (1961)

Brown, H.M., Cornwall, M.C.: Spectral correlates of a quasi-stable depolarization in barnacle photoreceptor following red light. J. Physiol. (Lond.) **248**, 555–578 (1975a)

Brown, H.M., Cornwall, M.C.: Ionic mechanism of a quasi-stable depolarization in barnacle photoreceptor following red light. J. Physiol. (Lond.) **248**, 579–593 (1975b)

Brown, J.E., Lisman, J.E.: An electrogenic sodium pump in *Limulus* ventral photoreceptor cells. J. gen. Physiol. **59**, 720–733 (1972)

Goldsmith, T.H.: The natural history of invertebrate visual pigments. In: Handbook of sensory physiology, Vol. VII/1 (ed. H.J.A. Dartnall), pp. 685–719. Berlin-Heidelberg-New York: Springer 1972

Hamdorf, K., Gogala, M., Schwemer, J.: Beschleunigung der "Dunkeladaptation" eines UV-Receptors durch sichtbare Strahlung. Z. vergl. Physiol. **75**, 189–199 (1971)

Hamdorf, K., Paulsen, R., Schwemer, J.: Photoregeneration and sensitivity control of photoreceptors of invertebrates. In: Biochemistry and physiology of visual pigments (ed. H. Langer), pp. 155–166. Berlin-Heidelberg-New York: Springer 1973

Hamdorf, K., Rosner, G.: Adaptation und Photoregeneration im Fliegenauge. J. comp. Physiol. **86**, 281–292 (1973)

Hamdorf, K., Schwemer, J.: Photoregeneration and the adaptation process in insect photoreceptors. In: Photoreceptor optics (eds. A.W. Snyder, R. Menzel), pp. 263–289. Berlin-Heidelberg-New York: Springer 1975

Hochstein, S., Minke, B., Hillman, P.: Antagonistic components of the late receptor potential in the barnacle photoreceptor arising from different stages of the pigment process. J. gen. Physiol. **62**, 105–128 (1973)

Horridge, G.A., Mimura, K.: Fly photoreceptors I. Physical separation of two visual pigments in *Calliphora* retinula cells 1–6. Proc. roy. Soc. B **190**, 211–224 (1975)

Horridge, G.A., Mimura, K., Tsukahara, Y.: Fly photoreceptors II. Spectral and polarized light sensitivity in the drone fly *Eristalis*. Proc. roy. Soc. B **190**, 225–237 (1975)

Kikuchi, R., Naito, K., Tanaka, I.: Effect of sodium and potassium ions on the electrical activity of single cells in the lateral eye of the horseshoe crab. J. Physiol. (Lond.) **161**, 319–343 (1962)

Koike, H., Brown, H.M., Hagiwara, S.: Hyperpolarization of a barnacle photoreceptor following illumination. J. gen. Physiol. **57**, 723–737 (1971)

Minke, B., Hochstein, S., Hillman, P.: Antagonistic process as source of visible-light suppression of afterpotential in *Limulus* UV photoreceptors. J. gen. Physiol. **62**, 787–791 (1973)

Minke, B., Wu, C.F., Pak, W.C.: Isolation of light-induced response of the central retinula cells from the electroretinogram of *Drosophila*. J. comp. Physiol. **98**, 345–355 (1975)

Muijser, H., Leutscher-Hazelhoff, J.T., Stavenga, D.G., Kuiper, J.W.: Photopigment conversions expressed in receptor potential and membrane resistance of blowfly visual sense cells. Nature (Lond.) **254**, 520–522 (1975)

Naka, K.: Recording of retinal action potentials from single cells in the insect compound eye. J. gen. Physiol. **44**, 571–584 (1961)

Nolte, J., Brown, J.E.: Ultraviolet-induced sensitivity to visible light in ultraviolet receptors of *Limulus*. J. gen. Physiol. **59**, 186–200 (1972)

Stark, W.S.: Spectral sensitivity of visual response alterations mediated by interconversions of native and intermediate photopigments in *Drosophila*. J. comp. Physiol. **96**, 343–356 (1975)

Stavenga, D.G.: Fly visual pigments. Difference in visual pigments of blowfly and dronefly retinula cells. J. comp. Physiol. **111**, 137–152 (1976)

Stavenga, D.G., Flokstra, J.H., Kuiper, J.W.: Photopigment conversions expressed in pupil mechanism of blowfly visual sense cells. Nature (Lond.) **253**, 740–742 (1975)

Tsukahara, Y., Horridge, G.A.: Visual pigment spectra from sensitivity measurements after chromatic adaptation of single fly retinula cells. J. comp. Physiol. **114**, 233–251 (1977)

Washizu, Y.: Electrical sensitivity of single retinula cells in the compound eye of the blowfly *Calliphora erythrocephala* Meig. Comp. Biochem. Physiol. **12**, 369–387 (1964)

Wulff, V.J., Mueller, W.J.: The origin of the receptor potential of the lateral eye of *Limulus*. In: The compound eye and vision of insects (ed. G.A. Horridge), pp. 37–52. Oxford: Clarendon Press 1975